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(54) Title: STEREOCHEMICAL WORTMANNIN DERIVATIVES

(57) Abstract

This invention relates to derivatives of Wortmannin and particularly to 11,17 substituted derivatives of Wortmannin, useful as PI-3-kinase inhibitors and as anti-tumor agents.

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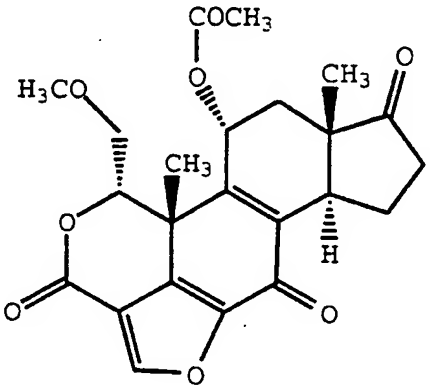
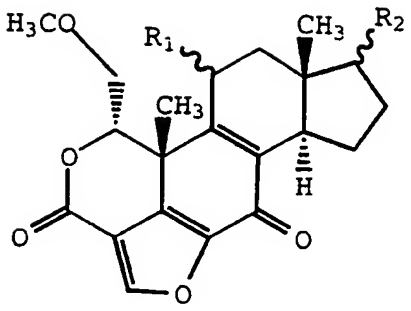
STEREOCHEMICAL WORTMANNIN DERIVATIVES

Wortmannin is a known potent inhibitor of phosphotidylinositol-3-kinase (PI-3-kinase), and has been suggested for use as a potential anti-cancer agent. Wortmannin is a naturally occurring compound isolated from culture broths of the fungus Penicillium wortmannin and has the following basic structure:



One of the disadvantages of wortmannin is its toxicity to living creatures. Even in low dosages, wortmannin in pure form was often lethal to laboratory animals. Attempts to synthesize derivatives of wortmannin have so far been problematical.

The present invention provides wortmannin compounds which exhibit enhanced potency for PI-3-kinase inhibition and have probable use as anti-cancer agents. The compounds of the present invention include 11-substituted, 17-substituted and 11, 17 disubstituted derivatives of wortmannin. Generally, these derivatives include 11-esters as their base substitution groups, but other like compounds will no doubt exhibit similar activity. The general formula for the compounds of this invention is:



(I)

wherein:

R_1 is $\overset{O}{\overset{\|}{}}$, or OR_3;

R_2 is $\overset{O}{\overset{\|}{}}$, or OR_3;

each R_3 individually is hydrogen, arylacyl, C_3-C_8 acyl or substituted acyl; and

when R_1 is $\overset{O}{\overset{\|}{}}$ or OH, R_2 is not $\overset{O}{\overset{\|}{}}$.

The present invention also provides pharmaceutical formulations which include the compound in combination with a pharmaceutically acceptable carrier, excipient or diluent. It also provides the use of the compounds as PI-3 kinase inhibitors, for example as anti-cancer agents.

The term "acyl" represents an alkyl group attached to a carbonyl group. Typical acyl groups include C_2-C_8 acyl groups such as acetyl, propionyl, butyryl, valeryl, isovaleryl and caprolyl.

The term "C_3-C_8 acyl" represents a C_2-C_7 alkyl group attached to a carbonyl group. Typical C_3-C_8 acyl groups include propionyl, butyryl, valeryl, isovaleryl and caprolyl.

The term "substituted acyl" represents a substituted alkyl group attached to a carbonyl group. Examples of substituents which may be present in a substituted alkyl group are halogen atoms, for example, chlorine; amino groups, for example dimethylamino; and alkylidene groups such as methylidene. Typical substituted acyl groups include substituted C_2-C_8 acyl groups such as N,N-dimethylaminopropionyl, acryloyl and chloroacetyl.

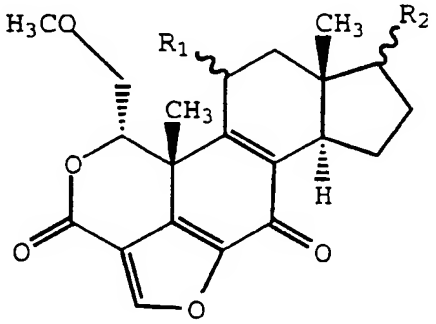
The term "arylacyl" represents an aryl or substituted

aryl group attached to an acyl group.

The term "aryl" represents an aromatic moiety, such as phenyl, and polynuclear aromatic moieties, such as naphthyl, fluorenyl, anthracyl and phenanthryl. The term "substituted aryl" represents an aryl group substituted with one or more moieties chosen from the group consisting of halogen, hydroxy, cyano, nitro, C_1-C_6 alkyl, C_1-C_4 alkoxy, carboxy, acetyl, formyl, carboxymethyl, hydroxymethyl, amino, aminoethyl or trifluoromethyl. Examples of substituted aryl groups include 4-methylphenyl, 2-methylphenyl, 4-methoxyphenyl, 4-(i-propyl)phenyl, 4-cyclopentylphenyl, 4-(1,1,4,4-tetramethylbutyl)phenyl, 4-acetylphenyl, 4-trifluoromethylphenyl, 4-chlorophenyl, 2-bromophenyl, 3-iodophenyl, 6-bromonaphthyl, 3,4-methylene-dioxyphenyl, indenyl, 1,2,3,4 tetrahydronaphthyl, and 1,2,4,4-tetramethyl-1,2,3,4-tetrahydronaphthyl. A typical value for an "arylacyl" group is phenylacetyl.

While all of the formula (I) compounds are believed to possess the ability to inhibit the action of PI-3-kinase, certain compounds are preferred. The preferred compounds have the general formula:

(Ia)



wherein R_1 is $\overset{O}{\overset{\|}{}}$, or OR_3;

R_2 is $\overset{O}{\overset{\|}{}}$, or OR_3; and

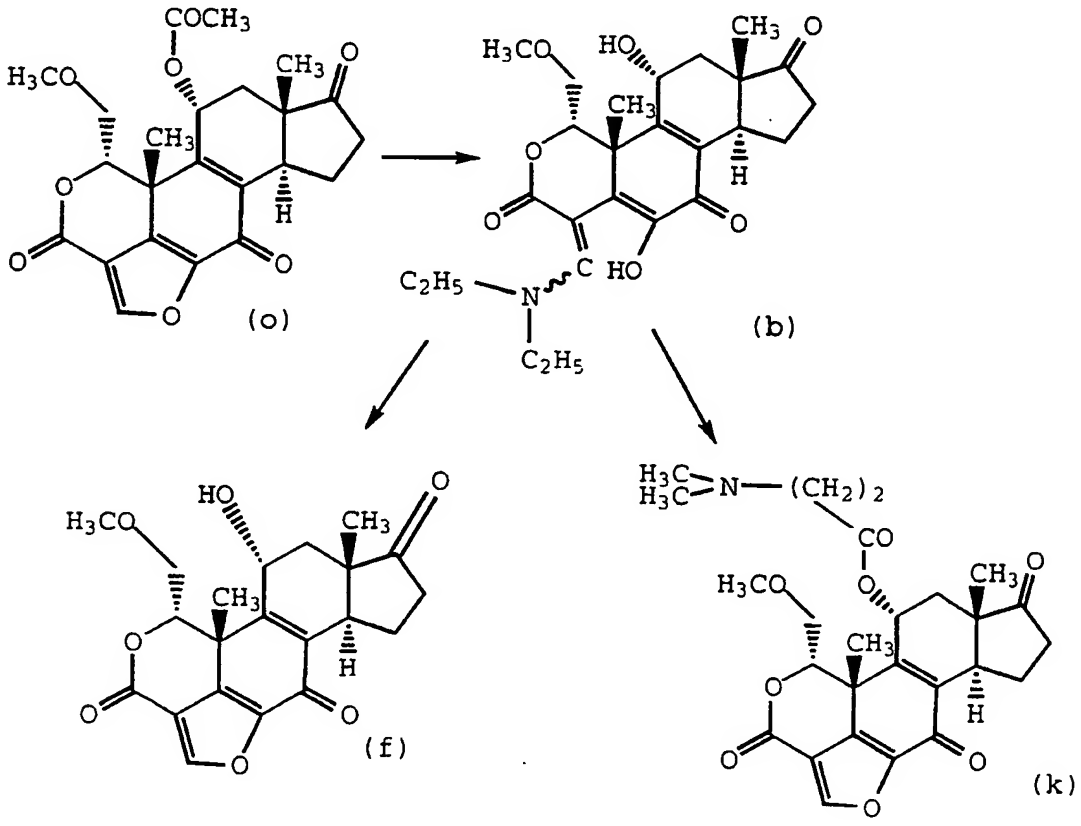
R_3 is hydrogen, C_3-C_8 acyl or substituted acyl.

In the most preferred compounds from this group of preferred compounds R_1=O-C_3-C_8 acyl or O-substituted C_2-C_8 acyl; and R_2=$\overset{O}{\overset{\|}{}}$, or OH, all of the compounds are

synthesized from wortmannin using procedures which will be described in detail below. It is understood that these procedures are merely indicative and introduced for purposes of explanation, not to be seen as limiting the invention to the steps and specific compounds described.

11-desacetyl derivatives of wortmannin are first prepared by methods well known in the art according to the following scheme I.

Scheme I



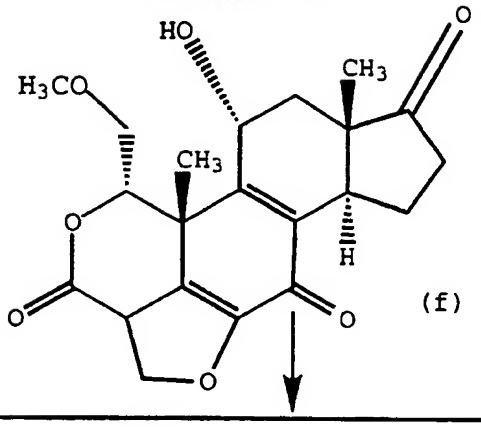
In the general scheme, wortmannin (o) is suspended in solvent and reacted with an amine to yield the open ring compound (b). Compound (b) generally does not show significant (IC_{50}>10ng/ml) activity as a PI-3-kinase inhibitor. Compound (k) is prepared from (b) by reaction with a tertiary amine and an acryloyl halide then with

dimethylamine, and reformation of the furan ring with a strong acid in solvent. Compound (f) is prepared by reacting compound (b) with a strong acid in the presence of a solvent. Purification of compounds (k) and (f) is carried out by well known methods.

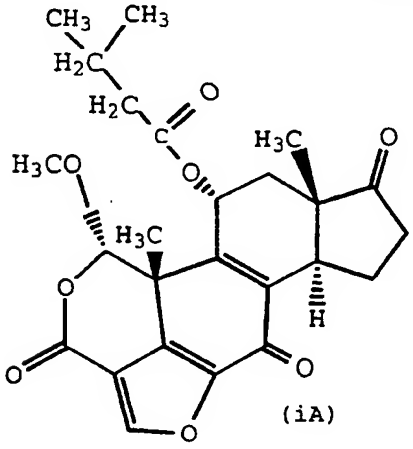
Compound (f) exhibits 50% inhibition vs. PI-3-kinase at 10ng/ml. The most preferred compounds are produced directly or indirectly from compound (f) according to the following Scheme II:

Scheme II

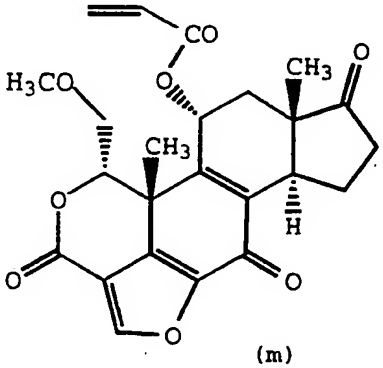
Scheme IIa



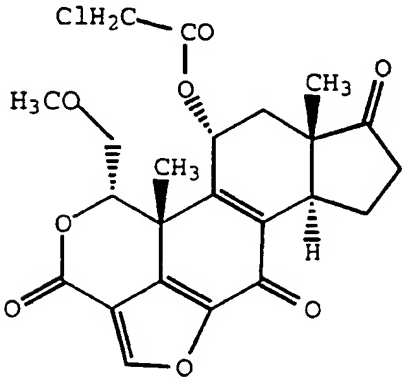
(f)



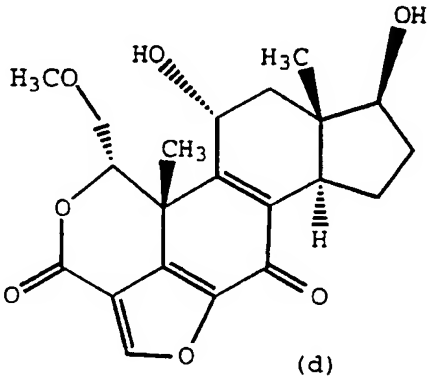
(iA)



(m)



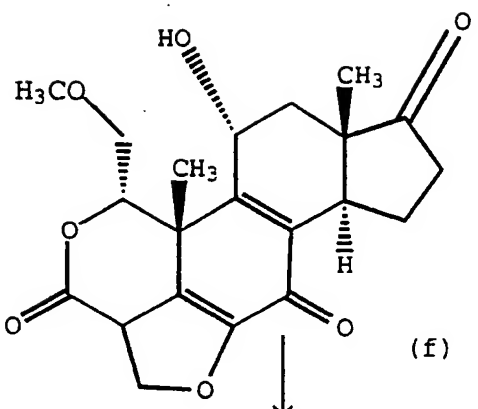
(l)



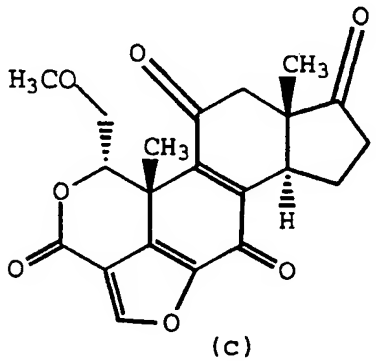
(d)

5

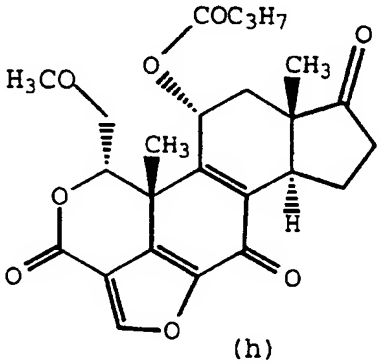
Scheme IIb



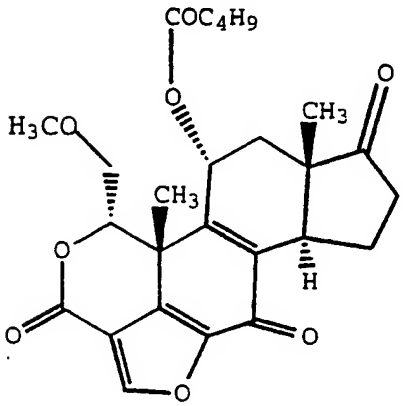
(f)



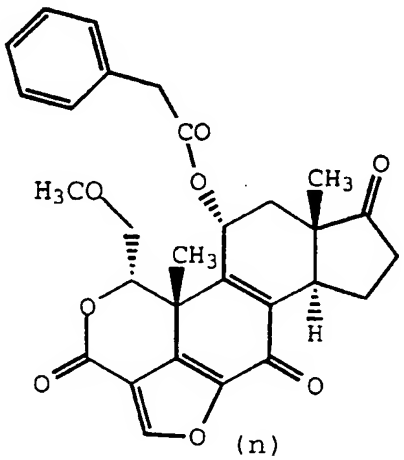
(c)



(h)

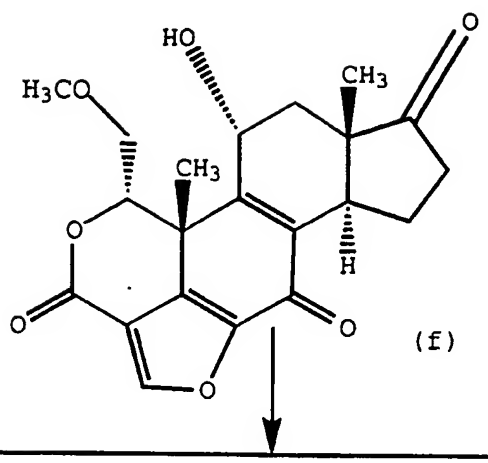


(i)

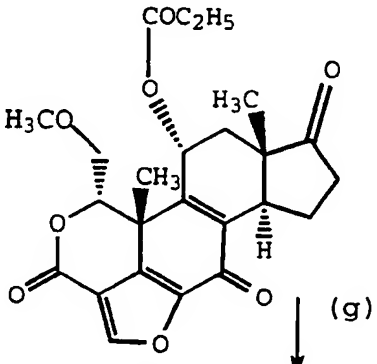


(n)

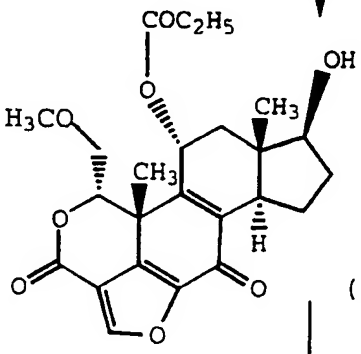
Scheme IIc



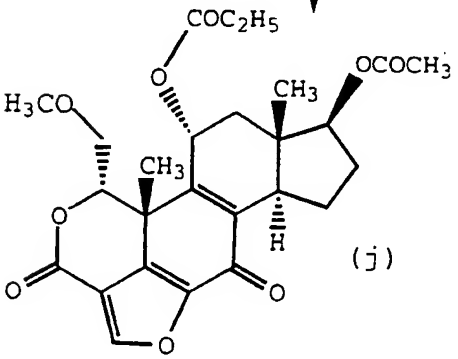
(f)



(g)



(e)



(j)

According to the above scheme, preferred compounds (g), (h) and (i) are prepared directly from compound (f) by reaction with the corresponding acid anhydride to produce the 11-substituted wortmannin esters. The isovaleryl derivative (iA) is prepared from (f) by reaction with an isovaleryl halide. Compound (c) is prepared by reacting (f) with an oxidizing agent to form the 11-oxy derivative. Compound (d) is prepared by reducing the 17-oxy group of (f) to a hydroxy.

Compound (n) is prepared by reacting (f) with a phenylacetoxy halide. Compound (l) is prepared by reacting (f) with a chloroacetyl halide, and (m) by reacting (f) with an acyloxyl halide.

Finally, compound (e) is formed by reduction of (g), and (e) may then be reacted with an acid anhydide to form compound (j). A detailed description of the procedures outlined above is presented later in this specification.

The present invention also provides for the use of the compounds as inhibitors of PI-3-kinase. In order to demonstrate the activity of the compounds of this invention, the following experiments were performed:

Purification of Phosphatidylinositol 3-Kinase

PI 3-kinase may be prepared by multiple methods. In one method, PI 3-kinase was prepared from confluent Swiss 3T3 cells obtained from the American Type Culture Collection, Rockville, MD. Prior to purification of PI 3-kinase, cells were maintained in bulk culture in Dulbecco's Modified Eagles Medium (DMEM; Sigma, St. Louis, MO) supplemented with 10% fetal calf serum and were passaged using 0.25% trypsin and 0.02% ethylenediaminetetracetic acid (EDTA). 24 x 10^6 cells on four, 100 mm culture plates were washed with 10 mL Hanks Balanced Salt Solution (HBSS; Sigma) pH 7.4, and the cells were left in DMEM without fetal calf serum for 1 hour before being stimulated for 15 minutes with 100 ng/mL of the recombinant human BB

homodimer of platelet derived growth factor (PDGF; Genzyme, Cambridge, MA). The medium was aspirated and the cells washed with 10 mL of HBSS before being lysed with 3 mL of 137 mM NaCl, 20 mM of Tris (pH 8.0) containing 1 mM of $MgCl_2$, 10% of glycerol, 1% of Triton X-100 (Rohm and Haas, Philadelphia, PA), 2 µg/mL of leupeptin, 2 µg/mL of aprotonin, 1 mM or phenylmethylsulfonyl fluoride (PMSF), and 1 mM of sodium orthovanadate. The cells were scraped free from the surface of the dish and centrifuged at 6,000 x g for 10 minutes. The supernatant was mixed with 50 µL of washed IgG2bk antiphosphotyrosine antibody beads (Upstate Biotechnology Inc., Lake Placid, NY) in 1.5 mL tubes. The tubes were capped and rotated for 2 hours at 4° C and the beads were twice washed with 1 mL of HBSS containing 2 µg/mL of leupeptin, 4 µg/mL of aprotonin, 1 mM of PMSF, 200 µM of adenosine, and 1 mM of sodium orthovanadate. The tyrosine phosphorylated PI 3-kinase was eluted from the beads with 200 µL/tube of 10 mM Tris (pH 7.5), 2 M of NaCl, 1 mM of EDTA, 200 µM of adenosine, and 10 mM of sodium phenylphosphate.

In another, preferred, method, PI 3-kinase was prepared from bovine brain. Two bovine brains (wet weight about 900 g) were obtained from a local slaughterhouse within minutes of slaughter, packed on ice, and homogenized within one hour. Brains were trimmed of excess fat and blood vessels and then homogenized using a Tekmar Tissuemizer (Cincinnati, OH) at 4°C in 20 mM of Tris(pH 8.3) containing 250 mM of sucrose, 6 mM of β-mercaptoethanol, 1 µg/ml of leupeptin, 1 µg/ml of pepstatin A, 0.4 mM of PMSF, and 1 mM of $MgCl_2$.

Following centrifugation for 60 minutes at 10,000 x g, the pH of the supernatant (about 1200 mL) was lowered to 5.75 using dropwise addition of 1M acetic acid at 4° C. After stirring for an additional 15 minutes at 4° C, the solution was centrifuged for 60 minutes at 13,500 x g. The supernatant was discarded. Pellets were resuspended in Buffer A (20 mM of Tris, pH 8.3, containing 6 mM of β-

mercaptoethanol, 0.1 mM of ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA), 1 μg/mL of leupeptin, 1 μg/mL of pepstatin A, and 1 mM of $MgCl_2$), and loaded onto a Fast Flow Q Sepharose column (300 ml) at a flow rate of 5 mL/min at 4° C. After loading, the column was washed with 3 volumes of Buffer A containing 0.1 M of KCl and the kinase was then eluted with a linear gradient of Buffer A/0.1M KCl to Buffer A/0.6 M KCl at 3 mL/min over 7 volumes.

Fractions were assayed for PI 3-kinase activity using 10 μL of the fraction and phosphatidylinositol as substrate as described below. PI 4-kinase eluted in the breakthrough; PI 3-kinase eluted at approximately 0.3 M of KCl. The PI 3-kinase pool was subjected to a 40% ammonium sulfate precipitation. Following centrifugation (60 minutes at 13,500 x g), pellets were resuspended in Buffer B (10 mM of potassium phosphate, pH 7.4, containing 6 mM of β-mercaptoethanol, 1 μg/mL of leupeptin, 1 μg/mL of pepstatin A, and 1 mM of $MgCl_2$), and loaded onto a 50 mL hydroxylapatite column (Calbiochem, Inc., La Jolla, CA) at 2.5 mL/minute. The column was washed with 150 mL Buffer B until the A_{280} baseline reached zero, and the kinase was then eluted with a linear gradient of 10-320 mM of KH_2PO_4 at 1 mL/minute over 450 minutes.

Active fractions were pooled and then loaded at 3 mL/minute onto a MonoS column (8 ml) (Pharmacia, Inc., Piscataway, NJ) equilibrated in Buffer C (50 mM of MES, pH 6.2, containing 6 mM of β-mercaptoethanol, 0.1 mM of EGTA, 1 μg/mL of leupeptin, 1 μg/mL of pepstatin A, and 1 mM of $MgCl_2$). PI 3-kinase was eluted with a linear gradient of 0-0.4 M KCl in Buffer C over 120 minutes. In assaying fractions, two pools of PI 3-kinase activity were routinely found. The bulk of the activity was found in the flow-through, while about 20% of the activity was eluted in the gradient. Although the material in the gradient had considerable PI 4-kinase activity, essentially no PI 4-kinase activity was associated with the PI 3-kinase eluted

in the flow-through. Therefore, the MonoS flow-through was concentrated by tangential flow filtration on a Mini-Ultrasette Omega 50 K membrane (Filtron, Inc., Northborough, MA) and diluted in Buffer C to lower the conductivity. The material was then reloaded onto the MonoS column using the above conditions. The PI 3-kinase bound to the column during the wash and was eluted in the gradient. Two pools of phosphatidylinositol kinase activity were obtained in the gradient; each was assayed for PI 3-kinase and PI 4-kinase activity. Pool I was found to contain 95% PI 3-kinase activity (and 5% PI 4-kinase) while Pool II contained predominantly PI 4-kinase activity.

Pool I from the MonoS column was diluted with Buffer A and chromatographed on MonoQ (1 ml) and eluted with a gradient of 0-0.4 M KCl in Buffer A. The final pool was assayed for PI 3-kinase and PI 4-kinase activity. The final product was found to contain greater than 99% PI 3-kinase activity.

Assay of Purified PI-3 Kinase Activity

PI 3-kinase activity was measured as previously described by Matter, W.F., *et al.*, Biochemical and Biophysical Research Communications, 186: 624-631 (1992). Inhibitor candidates were initially dissolved in DMSO and then diluted 10-fold with 50 mM of HEPES buffer, pH 7.5, containing 15 mM of $MgCl_2$ and 1 mM of EGTA. Ten microliters of this solution were incubated with purified bovine brain PI 3-kinase (9 µL) and phosphatidylinositol (5 µL of a 2 mg/mL stock solution in 50 mM of HEPES buffer, pH 7.5, containing 1 mM of EGTA). The final reaction mixture contained 0.1-5 ng/mL of inhibitor and 3% of DMSO (v:v). This concentration of DMSO had no effect on PI 3-kinase activity; control reaction mixtures contained 3% of DMSO (v:v) without inhibitor. Reactants were preincubated 10 minutes at ambient temperature and then the enzyme reaction was started upon addition of 1 µL [γ-^{32}P]ATP (2 mCi/mL, 500 µM of stock solution; 0.08 mCi/mL, 20 µM of final

concentration; Dupont New England Nuclear, Boston, MA). The reaction was allowed to proceed for 10 minutes at ambient temperature with frequent mixing, after which time the reaction was quenched by addition of 40 µL of 1N HCl. Lipids were extracted with addition of 80 µL $CHCl_3$:MeOH (1:1, v:v). The samples were mixed and centrifuged, and the lower organic phase was applied to a silica gel TLC plate (EM Science, Gibbstown, NJ), which was developed in $CHCl_3$:MeOH:H_2O:NH_4OH (45:35:8.5:1.5, v:v). Plates were dried, and the kinase reaction visualized by autoradiography. The phosphatidylinositol 3-monophosphate region was scraped from the plate and quantitated using liquid scintillation spectroscopy with ReadyProtein (Beckman Instruments, Inc., Fullerton, CA) used as the scintillation cocktail. The level of inhibition for wortmannin and analogs was determined as the percentage of [^{32}P]-counts per minute compared to controls.

Alternatively, products of the PI 3-kinase reaction were confirmed by HPLC as discussed by Whitman, M., Nature, 332: 644-646 (1988). Phospholipids were deacylated in methylamine reagent and separated using a Whatman Partisphere SAX anion exchange column as previously described by Auger, K.R., Cell, 57: 167-175 (1989). A Radiomatic Model A-140 Flo-One/Beta on-line radioactivity detector was used to monitor the deacylated [^{32}P]-enzyme products; deacylated [^{3}H]PI 4-monophosphate was added as an internal standard.

Based on these experiments the following IC_{50} values were obtained for inhibition of PI-3-kinase.

Compound	IC_{50} (ng/ml)
b	>10
c	1.6
d	>10
e	0.38
f	10
g	0.6
h	0.4
i (iA)	1.2 (1.16)
j	56.5
k	8.6
l	1.3
m	0.95
n	1.53

As can be seen from the above table, the compounds of this invention exhibit very potent activity as inhibiting agents of PI-3-kinase. Therefore, since PI-3-kinase activity has been linked to the formation and growth of various tumors, both benign and malignant, the compounds of this invention may also have usefulness as anti-tumor agents.

The present invention also provides for pharmaceutical formulations which include the above compounds and a pharmaceutically acceptable carrier, excipient or diluent. The following formulations are contemplated (active ingredient(s) refers to one of the wortmannin compounds of this invention):

Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
Active ingredient	250
Starch, dried	200
Magnesium stearate	10
Total	460 mg

Formulation 2

A tablet is prepared using the ingredients below:

	Quantity (mg/capsule)
Active ingredient	250
Cellulose, microcrystalline	400
Silicon dioxide, fumed	10
Stearic acid	5
Total	665 mg

The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3

An aerosol solution is prepared containing the following components:

	Weight
Active ingredient	0.25
Ethanol	29.75
Propellant 22 (Chlorodifluoromethane)	70.00
Total	100.00

The active compound is mixed with ethanol and the mixture added to a portion of the propellant 22, cooled to -30°C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

Formulation 4

Tablets, each containing 60 mg of active ingredient, are made as follows:

Active ingredient	60 mg
Starch	45 mg
Microcrystalline cellulose	35 mg
Polyvinylpyrrolidone (as 10% solution in water)	4 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1 mg
Total	150 mg

The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The aqueous solution containing polyvinylpyrrolidone is mixed with the resultant powder, and the mixture then is passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. Sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation 5

Capsules, each containing 80 mg of active ingredient, are made as follows:

Active ingredient	80 mg
Starch	59 mg
Microcrystalline cellulose	59 mg
Magnesium stearate	2 mg
Total	200 mg

The active ingredient, cellulose, starch, and magnesium stearate are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation 6

Suppositories, each containing 225 mg of active ingredient, are made as follows:

Active ingredient	225 mg
Saturated fatty acid glycerides	2,000 mg
Total	2,225 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

Formulation 7

Suspensions, each containing 50 mg of active ingredient per 5 ml dose, are made as follows:

Active ingredient(s)	50 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mL
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified water to total	5 mL

The active ingredient is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor and color are diluted with a portion of the water and added, with stirring. Sufficient water is then added to produce the required volume.

Formulation 8

An intravenous formulation may be prepared as follows:

Active ingredient	100 mg
Isotonic saline	1,000 mL

Example 1

Preparation of Compound (b)

To a solution of 12.13 grams of wortmannin in 700 ml of methanol was added 70 ml of diethylamine. This mixture was stirred for 22 hours at room temperature, then evaporating under reduced pressure at room temperature to form the open ring compound (b).

Example 2

Preparation of 11-desacetyl wortmannin (Compound (f))

After evaporation, the crude solid of (b) was dissolved in 900 ml of dioxane followed by addition of 240 ml of 1N HCl stirred for 19 more hours. The mixture was concentrated under reduced pressure, then diluted with water, and the aqueous layer extracted with dichloromethane. The dichloromethane was dried over sodium sulfate and evaporated at room temperature under reduced pressure to yield 12.2 grams of crude Compound (f). This crude compound was purified by chromotography on silica eluting with 25% hexane in ethyl acetate. 7.35 grams (67% yield) of the title compound was obtained as a yellow glass. Analysis for $C_{21}H_{22}O_7$-calculated:-65.28;H-5.74;found:
C-65.54;H-5.81.

Example 3

Preparation of 11-dimethylaminopropionyl-desacetyl-Wortmannin

To a solution of 252.9 mg of Compound (b) in 10 ml of anhydrous dichloromethane was added 480 µl of diisopropylethylamine followed by 223 µl of acryloyl chloride and stirred to room temperature for 3 hours. The solvent was evaporated under reduced pressure to yield a sticky orange solid. This crude material was cooled to 5°C in an ice/acetone bath. Ice cold dimethylamine (5 ml) was added to the mixture and stirred at -5°C for 1.25 hours.

The mixture was allowed to warm at room temperature and the dimethylamine was distilled off. The residue was separated by chromatography on silica eluting with 10% methanol in dichloromethane, then 20% methanol in dichloromethane in a one-step gradient. Two UV active products were isolated and the product with the lower R_f value was dissolved in 11 ml of dioxane and 2.3 ml of 1N HCl and the mixture stirred at room temperature for 20 hours. This mixture was diluted with ethyl acetate and washed with saturated sodium hydrogen carbonate. The organics were separated and combined, then washed with brine, dried with sodium sulfate, and evaporated at room temperature under reduced pressure. The crude product was purified by chromatography on silica, eluting with 7% methanol in dichloromethane. 37.4 mg of the title compound (14% yield) was isolated as a light orange solid.

Example 4

Preparation of 11-propionyl-desacetyl- Wortmannin

To a solution of 483.6 mg of compound (f) in 25 ml of pyridine was added 680 µl of propionic anhydride and the mixture stirred at room temperature for 26 hours. The mixture was evaporated at room temperature under reduced pressure and the residue purified by chromatography on silica, eluting with 50% hexane in ethyl acetate. This yielded 512.8 mg of the title compound (93% yield) as an off-white solid. Analysis for $C_{24}H_{26}O_8$-Calculated-C:65.15;H:5.92;Found-C:65.07;H:5.96.

Example 5

Preparation of 11,17-desacetyl-dihydro Wortmannin

100 mg of compound (f) was reacted with 250 µl of 1M Borane in 3.5 ml of anhydrous tetrahydrofuran under a nitrogen atmosphere, was added 250 µl of 1M Borane, and the mixture stirred at 0°C for 2.5 hours. The reaction was quenched by adding 1 ml of water at 0°C, then allowed to warm to room temperature, diluted with water and extracted

with ethyl acetate. The ethyl acetate was washed with brine, dried with sodium sulfate and then washed and purified to yield 65.8 mg of the title compound (65% yield) as a white solid.

Example 6

Preparation of

11-propionyl-17-acetyl-desacetyl-dihydro- Wortmannin

To a solution of 104.9 mg of compound (e) produced in Example 13, in 5 ml of pyridine was added 95 µl of acetic anhydride. The mixture was stirred at room temperature for 27 hours. The mixture was then evaporated and the residue purified by chromatography on silica, eluting with 40% ethyl acetate in hexane to yield 89.9 mg of the title compound (77% yield) as a white solid. Analysis for $C_{26}H_{30}O_9$-Calculated-C:64.19,H:6.22-Found-C:64.43;H:6.31.

The compounds prepared in Examples 7-14 were all prepared directly from compound (f), (11-desacetyl Wortmannin).

Example 7

Preparation of 11-phenylacetyl-desacetyl- Wortmannin

To a solution of 100.2 mg of (f) in 8 ml of anhydrous dichloromethane was added 150 µl of diisopropylethylamine followed by 120 µl of phenylacetyl chloride, and the mixture stirred for 23 hours. The mixture was diluted with dichloromethane and washed with saturated sodium hydrogen carbonate. The dichloromethane was washed with brine, dried with sodium sulfate and evaporated under reduced pressure to yield 214.6 mg of crude product which was purified by chromatography on silica, eluting with 50% hexane in ethyl acetate. 81.4 mg of the title compound (62% yield) was recovered as a light yellow glass.

Example 8

Preparation of 11-acrylyl-desacetyl- Wortmannin

476.7 mg of compound (f) was reacted with acryloyl chloride as described in Example 7, and purified as described to yield 470 mg of the title compound (87% yield) as a light yellow solid. Analysis for $C_{24}H_{24}O_8$-Calculated-C:65.45;H: 5.49 -Found-C:65.19;H-5.70.

Example 9

Preparation of 11-chloroacetyl-desacetyl- Wortmannin

442.8 mg of compound (f) was reacted with chloroacetyl chloride as described in Example 7. After washing and purifying 200.1 mg of the title compound (38% yield) was obtained as a light beige solid. Analysis for $C_{23}H_{23}O_8Cl$-Calculated-C:59.68;H:5.01-Found-C:59.66;H:5.06.

Example 10

Preparation of 11-isovaleryl-desacetyl- Wortmannin

479.9 mg of compound (f) was reacted with isovaleric chloride as described in Example 7. After washing and purifying, 350.3 mg of the title compound (60% yield) was obtained as a light orange solid.

Example 11

Preparation of 11-valeryl-desacetyl- Wortmannin

454.1 mg of compound (f) was reacted with valeric anhydride as described in Example 4, then purified to yield 517.7 mg of the title compound (93% yield) as a white solid. Analysis for $C_{26}H_{30}O_8$-Calculated-C:66.37;H:6.43-Found-C:66.53;H:6.60.

Example 12

Preparation of 11-butyryl-desacetyl- Wortmannin

457.5 mg of compound (f) was reacted with butanyl anhydride as described in Example 4, then purified to yield 486.6 mg of the title compound (90% yield) as a white

solid. Analysis for $C_{25}H_{28}O_8$-Calculated-C:65.78;H:6.18-Found-C:65.52;H:6.38.

Example 13

Preparation of

11-propionyl-17-hydroxy-desacetyl-dihydro- Wortmannin

To an ice cold solution of 345 mg of compound (g) produced in Example 4 above, in 3.5 ml of anhydrous tetrahydrofuran under a nitrogen atmosphere, was added 250 µl of 1M Borane, and the mixture stirred at 0°C for 2.5 hours. The reaction was quenched by adding 1 ml of water at 0°C, then allowed to warm to room temperature, diluted with water and extracted with ethyl acetate. The ethyl acetate was washed with brine, dried with sodium sulfate and evaporated and purified as above described to yield 309.9 mg of the title compound (89% yield) as a light yellow solid. Analysis for $C_{24}H_{28}O_8$:Calculated-C:64.85;H:6.35-Found-C-64.65;H-6.38.

Example 14

Preparation of 11-dehydro Wortmannin

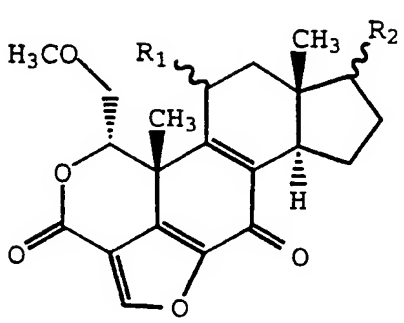
To a solution of 401.1 mg of compound (f) in 25 ml of anhydrous dichloromethane was added 1.96 grams of pyridinium dichromate and the mixture stirred for 2.5 hours. The mixture was filtered through celite, and the celite washed with fresh dichloromethane. The dichloromethane was combined and evaporated at reduced pressure and the crude product purified by chromatography on silica, eluting with 50% hexane in ethyl acetate to yield 313.9 mg of the title compound (79% yield) as an off white solid. Analysis for $C_{21}H_{20}O_7$-Calculated-C: 65.62;H:5.24-Found-C:65.42;H:5.33.

The above examples are to be viewed only as potential methods of producing the compounds of this invention, and not as limiting of the compounds in any way. Other compounds of the general formula (I) may be produced

utilizing one of the procedures outlined above and modifying that procedure in a well-known manner. It is foreseen that a person of ordinary skill in the art could easily produce any of the Formula (I) compounds by simply following one of the general schemes above.

We Claim:

1. A compound of the formula:



(I)

wherein:

R_1 is $\overset{O}{\overset{\|}{}}$, or OR_3;

R_2 is $\overset{O}{\overset{\|}{}}$,, or OR_3;

each R_3 individually is hydrogen, arylacyl, C_3-C_8 acyl or substituted acyl; and

when R_1 is $\overset{O}{\overset{\|}{}}$ or OH, R_2 is not $\overset{O}{\overset{\|}{}}$.

2. The compound of Claim 1 wherein:

R_1 is O-acyl and

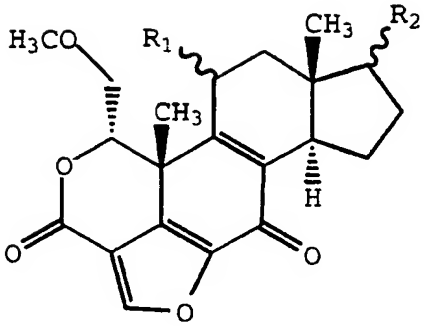
R_2 is $\overset{O}{\overset{\|}{}}$.

3. The compound of Claim 1 wherein R_1 is OR_3.

4. The compound of Claim 3 wherein R_3 is C_3-C_8 acyl or substituted acyl.

5. A compound of Claim 1 having the general formula:

(Ia)

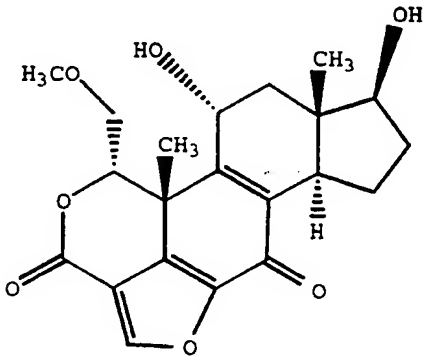


wherein R_1 is O-C_3-C_8 acyl, or O- substituted C_2-C_8 acyl; and

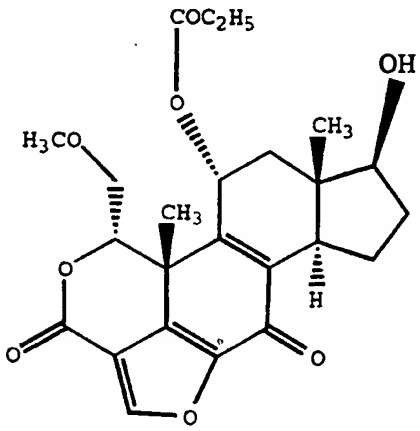
R_2 is $\overset{O}{\overset{\|}{}}$ or OH.

6. The compound of Claim 5 wherein R_1 is O-C_3-C_8 acyl.

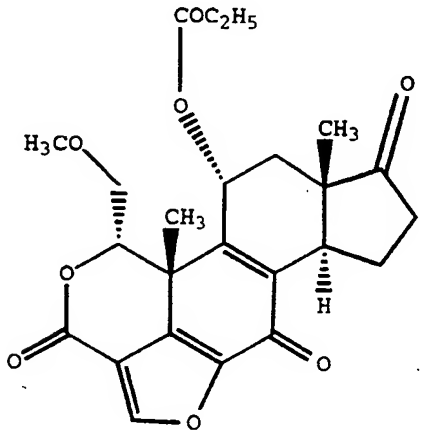
7. A compound of Claim 1 having the formula:



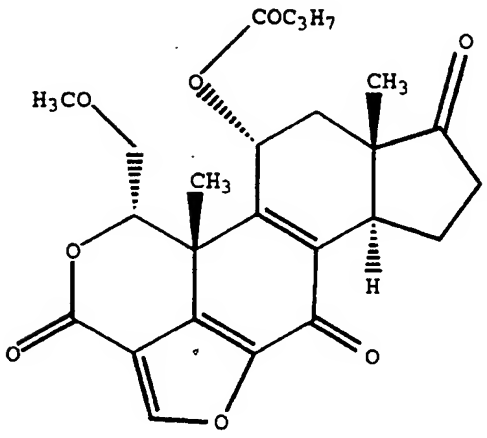
8. A compound of Claim 1 having the formula:



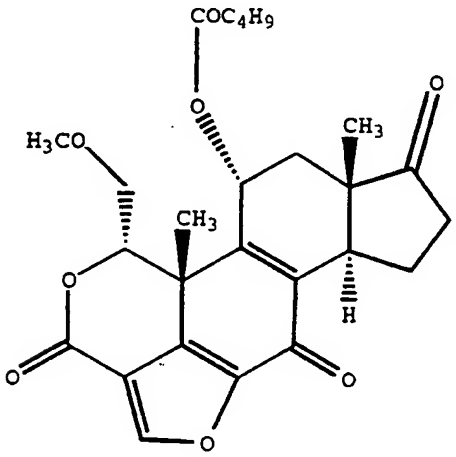
9. A compound of Claim 1 having the formula:



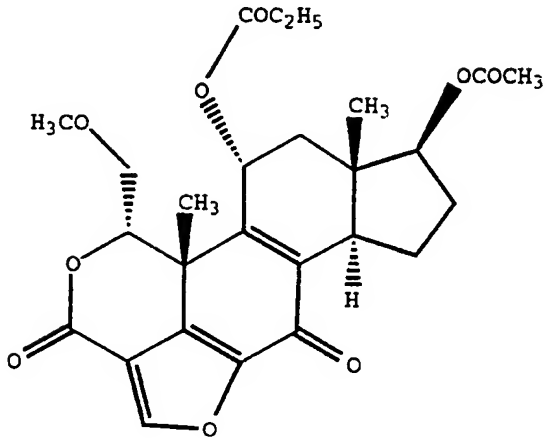
10. A compound of Claim 1 having the formula:



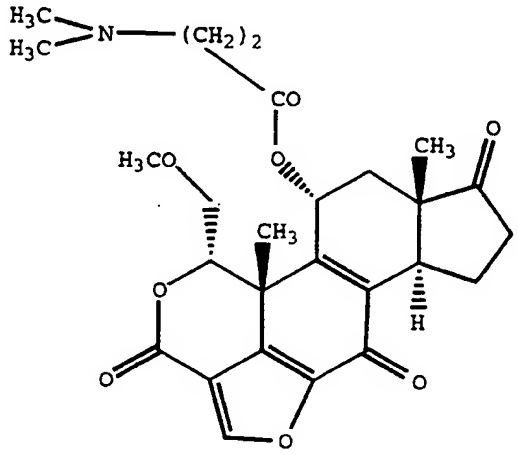
11. A compound of Claim 1 having the formula:



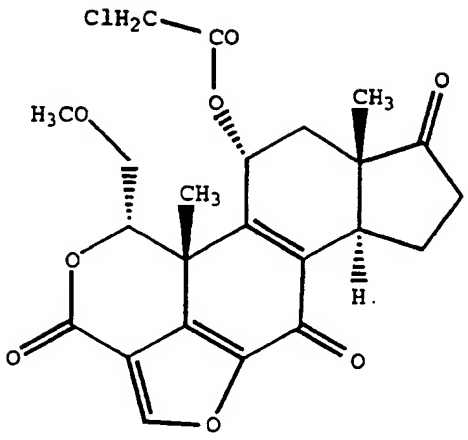
12. A compound of Claim 1 having the formula:



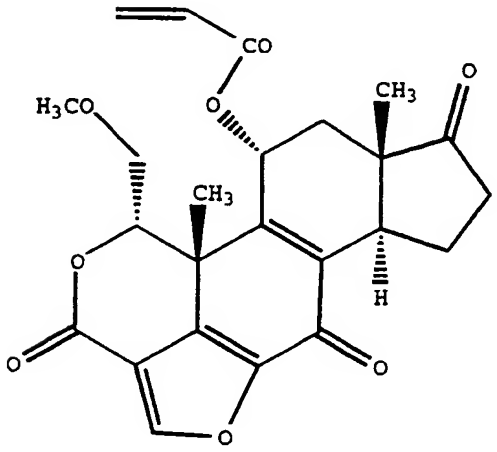
13. A compound of Claim 1 having the formula:



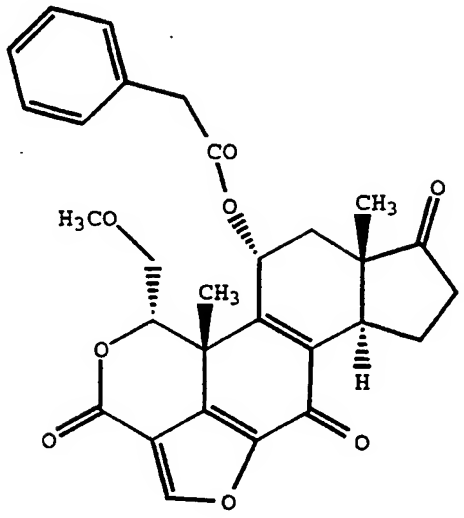
14. A compound of Claim 1 having the formula:



15. A compound of Claim 1 having the formula:



16. A compound of Claim 1 having the formula:



17. A pharmaceutical formulation comprising an effective amount of the compound of Claim 1, and a pharmaceutically acceptable carrier, diluent, or excipient.

18. The use of a compound as claimed in Claim 1 for the manufacture of a medicament for use as a PI-3 kinase inhibitor.

19. The use of a compound as claimed in Claim 1 for the manufacture of a medicament for the treatment of cancer.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08410

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/35; C07D 311/94
US CL :514/453; 549/275
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/453; 549/275

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY, Volume 268, Number 34, issued 05 December 1993, YANO et al., "Inhibition of Histamine Secretion by Wortmannin through the Blockade of Phosphatidylinositol 3-Kinase in RBL-2H3 cells", pages 25846-25856.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
28 AUGUST 1995	21 SEP 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer BA K. TRINH jd Telephone No. (703) 308-1235

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